CLINICAL LABORATORY BULLETIN May 2004

Web page: http://health.utah.gov/els/labimp

❖ INTRODUCING:

Michael Cuthbert	Chemistry
Dave Fredrickson	Chemistry
Boyd Nielsen	Chemistry
Chantelle Riddle	Client Services
Amber Scheid	Toxicology
Clarice Tappen	Chemistry

NOTEWORTHY

✓ **Rural Hospital Labs Test for Antimicrobial Resistance:** Utah is one of four states with labs who participated in a survey on methods and ability of rural hospitals to detect resistance in common bacteria. The survey pointed out rural labs have need of equipment and technical assistance to increase their effectiveness. The entire report is in *Diagnostic Microbiology and Infectious Diseases* (2003: 47:303-311).

✓ **Pre-analytic: What To Do With Those Routine Bacterial Culture Specimens Until Testing:** Every culture test result needs to be in the clinician's hand sooner. More and more laboratory work is being centralized to save money. What can you do ensure sample integrity during transport without compromising accuracy and patient care?

Transport requirements vary according to the testing method used. Check with your reference lab for their specific requirements. Research is showing some of our old notions on what **has** to be done to specimens for proper transport need changing. For example:

Any body fluid can be cultured in a blood culture bottle, inoculated at the bedside, as long as there is not a >24 hour delay in reaching the testing lab.

Respiratory specimens should be kept at 4° C and plated within 12 hours. Sputum specimens can be transported on swabs held at 4° C with <10% organism loss.

Urines with or without preservative held at 4° C and cultured within 24 hours will give an accurate colony count.

Feces is stable in commercial transport media. If you are only interested in *Salmonella* and *Campylobacter*, you can transport in saline at 4° C.

✓ ***C. psittaci* is still *C. psittaci*, or is it?** *Chlamydia* now have 2 genera of clinical importance: *Chlamydia* (includes *trachomatis*, *muridarum* and *suis*) and *Chlamydophilia*

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(includes *pneumoniae, pecorum, psittaci, abortus, caviae* and *felis*).

Taxonomists still have a job.

✓ **Troponin I by any other name is?:** Linda C. Rogers, PhD of Beckman Coulter, Inc. wrote an article in the January, 2004 MLO titled "The standardization of Troponin I assays: an update". In the article Dr. Rogers states there are about 20 assays available. Checking on proficiency test results for the same sample, one can find nearly a one-hundred fold difference from method to method. How can a clinician know what to do with the Troponin I assay results?

The American Association for Clinical Chemistry (AACC) created the cTnI Standardization Committee to create "harmonization" between methods (much like the INR tried to do for coagulation testing). The results of "round robin" testing from reference labs using various methods is available from 2001 and 2002. The committee hopes to create a reference standard that manufacturers can use to calibrate their methods and keep the results in "harmony" with all other methods.

This product, produced by the National Institute of Standards and Technology, should be available to manufacturers in 2004. Ask your manufacturer if they are using the product to standardize the assay you use.

✓ **Vancomycin Resistant Enterococcus (VRE) to or from the dogs?:** Dr. S. Simjee and colleagues wrote an article in 2002 for the *Journal of Clinical Microbiology* regarding genetic analysis of vancomycin resistance in human and animal enterococcal isolates. The transposon Tn5281 is often implicated in containing the resistance modification factors (on aac6' – aph2" genes).

The *van*A resistance locus on these genes has been found in community sewage, animal feces and raw meat. During investigation of enterococcal isolates from dogs at Michigan State University Veterinary Teaching Hospital, the authors discovered an isolate resistant to vancomycin and gentamicin. From sequence analysis the authors determined the isolate to be the same as isolates only found in human VRE.

This is the first reported case of VRE from a "companion" animal in the United States.

So who gave what to whom?

✓ **Fingersticks are wonderful for the patient and easy for the phlebotomist - unless:** If you have to squeeze the finger to get enough blood for a test, you will probably dilute the specimen with enough tissue fluid to give falsely low test results. You may also hemolyse the specimen. Even if the hemolysis is undetected, it can affect test results. If your method is not affected directly by hemolysed blood interfering, the extra liquid from the cells lysing will dilute your specimen. Whole blood methods require very small sample volumes and are greatly affected by small amounts of diluting fluid. Pre-warming collection sites before you collect capillary blood will increase flow as much as sevenfold. Better blood flow = less hemolysis and contaminating tissue fluids in your sample.

✓ **Discard tubes for multiple plastic tube draws?** The current recommendations for preventing carryover in coagulation apply to special factor assays only. You no longer need a discard tube for prothrombin and PTT tests.

If you are using safer, plastic tubes in a multi-tube draw, use a glass red top or a plastic blue top as a discard before drawing the coagulation factor assay tube. No tubes with additives (plastic red/yellow or speckled top) please as they can carry the clot activator to your blue top tube.

✓ **Monitoring Warfarin Therapy:** Brenda Katz, MD and Marisa B. Marques, MD wrote an article in the March issue of MLO on point of care, whole blood prothrombin time (P.T.) testing. The following highlights from their article may be of interest.

INR is established to aid clinicians in monitoring patients on warfarin therapy only. If the monitoring is done in the primary care physician's office, the patients are within range about 33% of the time. If the monitoring is done and patients followed in an anticoagulation clinic, they are in therapeutic range between 65% and 80% of the time.

There are currently three methods by which point of care instruments detect capillary blood clotting: optical blood flow detection, iron oxide particle oscillation, or peristaltic sample movement. Some instruments accept only capillary whole blood, others accept venous blood and some even accept citrated plasma. Instruments accepting citrated plasma can be calibrated to reference laboratory methods.

Hematocrits up to 56 as well as high platelet counts do not affect point of care PT results. Most instruments studied produce accurate results except for a positive bias at the high end of the INR scale when compared to reference results in one study of nine POC instruments. However another study asking physicians to determine dosing based on POC results led to a 22% unjustified warfarin dose increase. Read published studies carefully. There seems to be a "study" difference between instrument precision data and clinical decision. This must have something to do with the way statistical analysis alters the total view, but not the imprecision of individual test results.

An interesting study from Germany followed 3,000 patients with prosthetic heart valves. With patient self-monitoring, 78.3% of the time they were within therapeutic range versus 60.5% of the time when followed by their general practitioner.

✓ **Kudos - ARUP:** Congratulations ARUP for making the Fortune magazine's "The 100 Best Companies to Work For." the second year in a row.

✓ **Testing Method Interference:** You have the perfect instrument / method for the test you want to do. Employees are well trained in how to do the test. Samples are collected properly onsite and delivered immediately for testing. What could possibly go wrong? Ohhh . . . The November 2003 issue of CAP Today listed numerous things you need to take into account.

Julian H. Barth, MD, FRCP, MRC-Path, addressed the subject during last year's American Association for Clinical Chemistry national meeting. Dr. Barth is a consultant in chemical pathology and metabolic medicine at Leeds General Infirmary in Great Britain. Dr. Barth told his audience "Nobody knows what the problems with gel separators are and whether they interfere. When you ask the companies, they'll compare their gel separator tubes with somebody else's gel separator tubes. They don't compare them with glass tubes."

During a national external quality assurance test a second trimester pregnancy sample was distributed to British labs. They were asked to do LH. Five methods from three different manufacturers showed elevated amounts of LH. Like the cases in the USA when labs were successfully sued, you could diagnosis polycystic ovaries when all you had was a sample from a normal pregnant woman.

One digoxin method is negatively inhibited by spironolactone and canrenoates (common drugs used for patients in ICU).

Dr. Barth told of a patient with E. coli sepsis who had symptoms of heart attack. The troponin assay was elevated. Other tests (ECG, echo, angiogram) were normal as the troponin continued to rise. The serum was tested on

numerous troponin assays. Some results were elevated, some were not. The patient's own antibodies interfered with some methods, but not all methods.

Even when the test result fits with the diagnosis, there may be interference. If you use a secondary method as back up, be sure to study how it compares with the primary method very carefully. There can be interference with one and not the other.

Dr. Barth cautions, "Just because somebody has normal results doesn't mean they haven't got interference." "And my personal approach is always to get a fresh sample, because often the first sample that comes through with a funny result is a funny sample."

It seems closer lab contact with the clinician could save some patients a lot of unnecessary expense, time and trauma.

FROM THE PATIENT'S CHART

"She is numb from her toes down."

☆ Feature ☆

CHECK YOUR D-DIMER IQ

Sterling Bennett, MD, from LDS Hospital Pathology Department gave the following quiz at the end of his talk on D-dimer assays April 2, 2004 during the USCLS Spring Seminar. With Dr. Bennett's permission, test your knowledge of D-dimer. Answers are on the last page of this issue.

1. D-dimer is a degradation product of:
   A. Fibrinogen
   B. Fibrin monomers
   C. Cross-linked fibrin
   D. All of the above

2. Elevated D-dimer indicates that thrombi are pathological.
   A. True
   B. False

3. The "gold Standard" method for D-dimer testing is considered to be:
   A. Latex agglutination
   B. ELISA
   C. Hemagglutination
   D. Immunoturbidimetry

4. Some commercially available D-dimer assays have sensitivity equal to ELISA tests.
   A. True
   B. False

5. What is the most serious DVT complication?
   A. Leg pain
   B. Leg swelling
   C. Clot extension
   D. Pulmonary embolism

6. More than 90% of venous thromboembolism cases can be diagnosed by clinical examination.
   A. True
   B. False

7. D-dimer is a desirable test for VTE evaluation for each of the following reasons except:
   A. Indicates presence of VTE
   B. Inexpensive
   C. Short turnaround time
   D. Readily available

8. The likelihood that a patient does not have VTE when the D-dimer test is negative is called:
   A. Sensitivity
   B. Specificity
   C. Positive predictive value
   D. Negative predictive value

9. A negative D-dimer effectively excludes the diagnosis of VTE.
   A. Yes
   B. No
   C. Maybe so

10. In general, quantitative assays have higher sensitivity for D-dimer than qualitative assays.
   A. True
   B. False

11. DIC may be defined as the systemic production of ___ and ___ in a suitable clinical setting.
   A. Hemorrhage and thrombosis
   B. Clotting factors and platelets
   C. Thrombin and plasmin
   D. D-dimer and schistocytes

12. Hemorrhagic manifestations of DIC are usually more noticeable than thrombotic manifestations, but thrombosis must be stopped to successfully treat DIC.
   A. True
   B. False

13. Each of the following is a major patho-physiologic mechanism of DIC except:
   A. Procoagulant activation
   B. Microvascular damage
   C. Factor depletion
   D. Inhibition of plasmin

14. Elevated D-dimer indicates all of the following except:
   A. DIC is present
   B. Thrombin has been generated
   C. Fibrin has been crosslinked
   D. Plasmin has been generated

15. Which type of D-dimer assay is most useful for DIC diagnosis?
   A. Quantitative
   B. Semi-quantitative
   C. Qualitative
   D. All are equally useful

16. Qualitative D-dimer tests have advantages over quantitative tests for monitoring DIC.
   A. True
   B. False

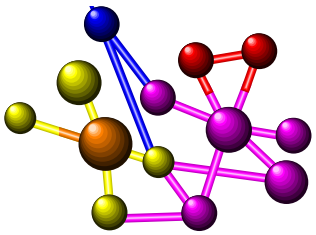
17. D-dimer assay sensitivity is more important for use in VTE than in DIC.
   A. True
   B. False

18. Of the following, the most reliable test for DIC diagnosis is:
   A. PT
   B. aPTT
   C. D-dimer
   D. Fibrinogen

19. I learned something new today (especially after reading the answers!)
   A. Yes
   B. No

Thank you Dr. Bennett

CLIA BITS



ADDITIONAL WAIVED TESTS:

° ACON *Helicobacter pylori* Rapid Test Device

° DE Healthcare Products TruView Strep A Cassette Test

° Abbott Medisense Precision Xtra Advanced Diabetes Management System

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NEW CLIA REGS – ALTERNATE VS EQUIVALENT QC

Two terms in the final CLIA regulations that may cause confusion or be overlooked are equivalent and alternate quality control (QC).

Equivalent QC (eQC) is a mechanism used to reduce the frequency of external controls (2 levels in most cases) from each day of testing to either weekly or monthly depending on the test system and results of the lab's evaluation for test accuracy. The process to follow for eQC is in the CLIA regulation guidelines at 493.1256.

Alternate QC (also found at 493.1256) is a "substitute" for commercially prepared control materials that are not available for some tests such as the newly developed methods used to identify bioterrorism organisms in blood samples. Another example is non-kit / instrument tests such as the KOH exam. You need to assess the accuracy of the test or procedure immediately and over time in differing environmental conditions you face during the year. You need to assess the test functions accurately with different personnel who may perform the testing. Alternate control methods include but are not limited to: splitting a sample to be tested with another method or at another lab; include previously tested patient specimens (tested in duplicate); test each specimen in duplicate, test multiple specimen types from the same patient (saliva, urine, serum); perform serial dilutions of positive specimens to confirm positive reactions; or provide additional supervisory review of results prior to release. As soon as calibration or QC materials become available the lab must use them instead of the alternate method.

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The 2004 National Committee on Clinical Laboratory Standards (NCCLS) updates for bacterial susceptibility testing quality control (QC) ranges include the following changes:

Minimal Inhibitory Concentration (MIC) QC ranges for oritavancin were added for *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, and *Streptococcus pneumoniae* ATCC 49619.

A footnote was added to the MIC table that includes QC ranges for ticarcillin-clavulanic acid for *Escherichia coli* ATCC 35218.

A footnote was added to both the disk diffusion and MIC tables specifying the QC ranges for amoxicillin-clavulanic acid and *Escherichia coli* ATCC 35218 when using *Haemophilus* Test Medium.

A footnote was added to the MIC table indicating testing amoxicillin and *Escherichia coli* ATCC 35218 on HTM may help determine whether the isolate maintained its ability to produce **$**-lactamase.

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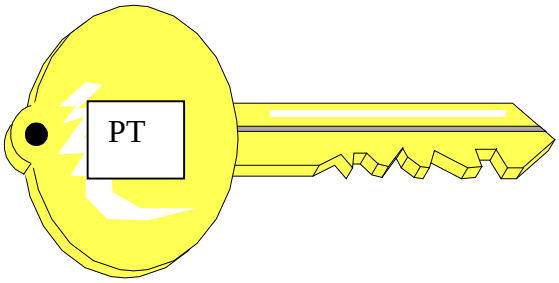
Barbara Harty-Golder, MD, JD wrote an opinion in the April 2004 issue of MLO stating HIPAA rules regarding patient confidentiality did not require a lab to obliterate slide labels before discarding the slides. Other research she cited states that while the slide may be traceable to a particular patient while the slide is still in the facility, the name itself could not give someone the confidential diagnosis. The results of a "diagnosis" derived from the slide is a different matter. The cost and safety issues required to destroy slides or slide labels does not justify the fear of identifying a confidential patient condition. Besides, **if your slides are labeled with a unique identifier instead of just the patient's name, all the better**!

Equals

"16.5 feet in the Twilight Zone: 1 Rod Sterling"



Sanctions were imposed on a Utah facility this year for improper proficiency testing (PT) communication. The facility had two separate offices. Each office lab was enrolled in the same PT program. One person received the results from both labs, completed the paper work and submitted the results to the PT provider. When the person noticed the two labs had different results for 2 of 5 specimens on one event, the testing personnel were asked to repeat their samples. Indeed, one lab had transposed the results for the 2 questionable samples. The "transposition error" was corrected, corrected results submitted to the provider, and a note counseling the employee was placed in the personnel file. Both labs scored 100% for the testing event.

This "communication" before submitting test results is just as serious as taking the specimens to another site to test. The law written by congress in 1988 states such communication results in immediate termination of both laboratories for a least 2 years.

What can a facility with 2 or more labs do to prevent such "communication"? The best solution is to have each lab order PT from a separate provider – hence no communication possible. OR - if each lab sends in their own results to the same provider independently and no one sees both results until after the closing date for each event, they may avoid changing results or redoing tests that do not match with the other lab. The first option is still preferable.

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Connie Laubenthal wrote an article for the February 2004 issue of MLO titled "Laboratory proficiency testing sports anomalies". The article had some good points such as the new CLIA regulation requires PT providers to accept results if 80% of their referees obtain consensus. The old rule allowed them to require 90% consensus to grade a result. Now more participant results should be graded.

The article mentioned that labs must now "verify the accuracy of any ungraded PT". In fact, the rule states the lab must "self-grade" results if the information to do so is provided. This was always part of the old CLIA quality assurance regulation. Only its placement in the regulations and exact wording has changed.

The author described situations that surveyors need to take into account when looking at PT results. Such information includes bias by different methods to an "all" methods lumped result; lumping of same instrument / methods that may not be the same based on a change in reagent formulation, etc. that some but not all labs have yet; and specimen matrix effect. CLIA surveyors are taught in various training sessions to take these and other anomalies into account before recommending any testing suspension due to faulty PT results. Also, surveyors usually give a lab 3 rather than the 2 failures provided in the CLIA regulations before imposing sanctions (providing the lab has been working to solve the problem). Most labs voluntarily stop testing an analyte or system that is not functionally properly since they want accurate test results for their clients.

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CAP has added D-dimer, CD34+, CD4+, CD8+ and quantitative rheumatoid factor, rubella and ASO to the analytes they will officially grade and report to CMS beginning in 2004.

SAFETY

Recall Notice: The Utah Public Health Lab (UPHL) received notice on April 2, 2004 from FDA of a recall for some lots of IMOVAX Rabies Vaccine. The vaccine is made by Aventis Pasteur in Swiftwater, Pennsylvania. The recalled lot numbers are X0667-2, X0067-3, W1419-2 and W1419-3. The UPHL had used the last lot number to immunize an employee who tests animals for rabies. So we know at least one of the affected lots was distributed in Utah.

It seems the company found one lot of vaccine made during the same time period as the 4 lots listed contained non-inactivated virus. This known affected vaccine lot was not distributed in the USA. Removing the other lots was a "precautionary" measure.

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As part of OSHA's Needlestick Safety and Prevention Act in effect since April, 2001 most labs are aware needles are not to be reused. Especially following a report from a large California lab a couple of years ago tracing HIV cases back to a phlebotomst who was cleaning and reusing needles to save the facility money, we know this practice can result in transmitting infection from one person to another. But what about the needle holders? John Henshaw, OSHA Administrator, states "Removing contaminated needles and reusing blood tube holders can pose multiple hazards." "Single-use blood tube holders, when used with engineering and work practice controls, simply provide the best level of protection against needlestick injuries. OSHA's bloodborne-pathogens standard specifically prohibits the removal of contaminated needles."

"To change and change for the better are two separate things."
Unknown

CONTINUING EDUCATION

CLIA BROCHURE #2 AVAILABLE

The second brochure in the series meant to help facilities understand the final CLIA regulations in now available. "Verification of Performance Specifications" is on the CLIA website. Log onto our site (location on page 1 of this bulletin) and under Clinical Laboratory Certification select CMS CLIA home page.

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IATA TRAINING

BLI was asked to sponsor an official IATA Training this year. Johnny Zonta from IATA will set up a one or two day training course in SLC to meet our needs. The training will meet IATA regulations to certify new persons in packaging and shipping infectious substances; meet the every two year recertification requirements for trained persons; and will update us on the impact of the new chemical terrorism sample transport plans.

The course can accommodate 25 persons. The cost would be $150 to $200 depending on the number of participants and course length.

If you are interested, please call Rebecca Christiansen at 801-584-8471 or Kim Christensen at 801-584-8400. This course has been well attended in other states, but this is our first opportunity to host it in Utah.

D-dimer IQ Answers

1. C	2. B	3. B
4. A	5. D	6. B
7. A	8. D	9. C
10. A	11. C	12. A
13. D	14. A	15. A
16. B	17. A	18. C

1999 British GCSE exam results from 16 year olds:

Q: Give an example of a fungus. What is a characteristic feature?

A: Mushrooms. They always grow in damp places and so they look like umbrellas.